Characterization of Bioactive Secondary Metabolite from Actinomycetes Against Biofilm Forming Bacterial Pathogens

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Abstract:

Biofilms are complex assemblages of microbial cells enclosed in a self-synthesized polymeric matrix. Bacteria growing as a biofilm remain a significant challenge in biomedical field, growing on abiotic material such as catheters and prosthesis as they tend to be more tolerant antimicrobial treatments. Current microbial inhibition strategies based on planktonic bacterial physiology have been known to have limited efficacy on the growth of biofilm communities. This problem is further intensified by the emergence of increasingly resistant strains. All aspects of biofilm measurement monitoring, dispersal control and inhibition are becoming issues of increasing importance. At present non-toxic biologically active compounds capable of inhibiting biofilms are interested. One of the attractive bioresources of novel bioactive compounds is actinomycetes as they variously produce potential antibiotics, enzymes, enzyme inhibitors and pharmacological agents. Several reports revealed that many actinomycetes produce anti-biofilm agents against different pathogenic bacteria. However, the effectiveness of many antimicrobial agents is currently decreasing due to prevalence of multidrug resistant pathogens. Therefore it is important to search for alternative therapeutics to control biofilm associated infections. In this study we proposed to characterize the bioactive metabolite produced by actinomycetes and examine its role in dispersion or inhibition of microbial biofilms.

Introduction

Biofilm is an assemblage of microorganisms enclosed in a matrix of extracellular materials such as extracellular polysaccharide (EPS), and relates to bacterial virulence, pathogenesis, and environmental survival. Formation of biofilm made cells more resistance to

stress, antibiotics, host immune response, and disinfectant compared to planktonic bacteria (Jiang et al., 2011). Biofilms consist mainly of extracellular polysaccharide (EPS), protein, and extracellular nucleic acid (Carpenter, 1971). The development of biofilm can be seen as a five stage process: initial reversible adsorption of cells to the solid surface, production of extracellular polymeric matrix substances resulting in an irreversible attachment, early development of biofilm architecture, maturation, and dispersion of single cells from the biofilm.

A bacterial cell initiates attachment to the surface by using EPS glycocalyx polymers and this first step is important in biofilm formation. In Gram-negative bacteria, glycocalyx polymers also form a rigid structure called a capsule or loose slime layer. Some Gram-positive bacteria also have a capsule, but it is more common in Gram-negative bacteria (Vidyawan and Waturangi, 2013).A majority of the biofilm formation involves cell-to-cell signaling using an autoinducer. This bacterial communication is called quorum sensing. There are several different quorum sensing autoinducers in bacteria. In Gram-negative bacteria the autoinducer compounds are acylatedhomoserine lactones (AHL/AI-1). In addition to AHL, 2-heptyl-3-hydroxy-4-quinolone (PQS) and diketopiperazines have also been detected in Pseudomonas aeruginosa and other bacteria (Pesci et al., 1999). In Gram-positive bacteria, peptides are used as autoinducers. Autoinducer 2 (AI-2) was recently found in both Gram-positive and Gram-negative bacteria (Vendeville et al., 2005).

Biofilm formation is also mediated by a number of mechanical, biochemical and genetical tools. The pili and flagella are generally involved as adhesive structures to help in attachment to biotic or abiotic surfaces. The role of an attachment factor, cellulose fiber and lipopolysaccharide (LPS) interactions to maintain strength and integrity in biofilm making in *Pseudomonas fluorescens* SBW25 has also been studied. The requirement of type IV pili has been implicated in maximal biofilm formation by *Clostridium perfringens* (Varga et al., 2008). Using the protection provided by the biofilm, pathogenic bacteria can more easily cause chronic infection in their host. If the bacterial colonies are not immediately separated from the surface, they can anchor themselves more permanently using cell-adhesion molecules. Therefore, searching for compounds orstrategies to decrease or destroy bacterial biofilm formation is essential.

There is an ever increasing amount of biofilm research aimed at understanding how bacteria control their biofilm formation and to discover non-toxic compounds that can inhibit or

disperse biofilm formation without allowing bacteria to develop drug resistance (Hentzer et al. 2003). The actinomycetes, including Streptomyces, are ubiquitous in the environment; they have been isolated worldwide from soil, ocean, and organic matter (McNeil and Brown 1994). Members of the Streptomyces family are a rich source of bioactive compounds, notably antibiotics, enzymes, enzyme inhibitors, and pharmacologically active agents.

Recently, a few reports have showed that actinomycetes contained active antibiofilm compounds against several pathogenic bacteria: for example, extracts of *Streptomyces albus* isolated from marine sources inhibited the biofilm formation of *Vibrio harveyi* (You et al. 2007), and methanol extracts of coral-associated *Streptomyces akiyoshiensis* inhibited biofilm formation of *Staphylococcus aureus* (Bakkiyaraj and Pandian 2010). *Rhodococcussp*.BFI 332 is also found to inhibit *Escherichia coli* O157:H7 biofilm formation (Lee et al. 2012) and that extracellular protease in actinomycetes culture supernatants inhibits and detaches *S. aureus* biofilm formation (Park et al. 2012). The overall goal of this study is to identify non-toxic and potent biofilm inhibitors from the high-throughput screening of actinomycetes strains against biofilm forming bacterial pathogens in order to understand the mechanistic basis of the biofilm inhibition.

2. Review of Literature and Development in the subject (Previous work done in the relevant area):

Kim et al.,(2012) studied antibiofilm activity of *Streptomyces* sp. BFI 230 and Kribbellasp BFI 1562 against *Pseudomonas aeruginosa* and indicated that actinomycetes strains have an ability to control the biofilm of *P. aeruginosa.*

Waturangi et al., (2016) reported that the bioactive crude extracts from actinomycetes has high potential in treating biofilm related infection caused by Gram positive and Gram negative pathogens.

Alasil et al., (2014) found that the purified extract derived from novel *Paenibacillussp are* nontoxic exhibiting strong antibiofilm activity against Gram positive and Gram negative pathogens that can be used for management.

Actinomycetes have provided many industrially important bioactive compounds having great economic importance and always being a curious organism for secondary metabolite production. Actinomycetes have long been recognized as prolific producers of enzymes, antibiotics, anti-cancerous agents and play important role in recycling of organic matter.In

natural niches, bacteria coexist in multispecies communities and compete with each other for resources and space. As a result, many bacteria have developed the ability to adapt to environmental niches. For example, actinobacteria produce antibacterials (Mahajan & Balachandran 2012), antifungals (Gupte et al. 2002), and a number of useful anti-tumor drugs. Recently, some actinomycete species were reported to produce *S. aureus* biofilm inhibitors. Methanol extracts of coral-associated *Streptomyces akiyoshiensis*(Bakkiyaraj& Pandian 2010), extracellular proteases from *Streptomyces* sp. BFI 250 and *Kribbella*sp. BFI 1562 (Kim et al. 2012), alnumycin D from *Streptomyces albus*, granaticin B from *Streptomyces violaceoruber*(Oja et al. 2015), and streptorubin B from *Streptomyces* sp. MC11204 (Suzuki et al. 2015) were shown to inhibit biofilm formation and also disrupt pre-established *S. aureus* biofilms.

Several reports revealed that actinomycetes produced anti-biofilm agents against different pathogenic bacteria. For example, extracts of *Streptomyces albus* inhibited biofilm formation of *Vibrio harveyi* (You et al., 2007); extracts of coralassociated *Streptomyces akiyoshiensis* inhibited biofilm formation of *Staphylococcus aureus* (Bakkiyaraj et al., 2010); *Rhodococcus* sp. BFI 332 inhibited *E. coli* O157:H7 biofilm formation (Lee et al.,2012); and extracellular proteases from marine actinomycetes inhibited and detached S. aureus biofilm formation (Park et al.,2012).

3. Objectives of Research:

- Isolation of Actinomycetes from different environments.
- Screening and Evaluation of bioactive metabolite producing actinomycetes for antibacterial and antibiofilm activity.
- Identification of promising isolate at molecular level.
- Production optimization and purification of bioactive metabolite.
- Characterization of bioactive compound.
- Quantification of its antibiofilm activity.

4. Methodology to be adopted:

1. The environmental samples including soil,water, agricultural fields and marine environments will be collected.
2. Actinomycetes isolation will be carried out on different nutrient medium .
3.Screening of isolated Actinomycetes will be carried out by using selective standard methods.
4. Detection of antimicrobial efficacy of bioactive metabolite against biofilmproducingBacteria
5. By using a specified medium production of bioactive metabolite will be carried out.
5. Purification and characterization of produced bioactive metabolite will be done by using analytical methods.
6. Quantification of biofilm inhibitory potential of bioactive compound.

5. Importance of study/ Society application:

International status:

At the international level,Lokegaonkar and Nabar (2017) reportedanti-biofilm, antibacterial, antiquroum sensing activity of gamma tolerant actinomycete isolated from soil. They obtained Secondary metabolites from *Streptomyces albogriseolus*GIS39Ama (genbank Accession number: KX694268) and estimated minimum biofilm inhibitory concentrations (MBIC) of metabolite against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Escherichia coli* and *Vibrio cholera.*

Sara et al.(2018) investigated the antimicrobial as well as the antibiofilm activity of papain enzyme on drug-resistant biofilm producing *Klebsiella Pneumoniae*.

National status:

At the national levelGovinadanRamchandran et al. (2018) isolated and identified antibacterial compound from marine endophytic actinomycetes against multi drug resistant bacteria.

Rajivgandh**i**et al. (2018) studiedantibiofilm activity of marine endophytic actinomycetes compound isolated from mangrove plant *Rhizophoramucronata*, Muthupet Mangrove Region, Tamil Nadu, India.

- **Significance of the study**

a. This study will be helpful in enhanced production of bioactive compound. The present study may contribute to isolation of new actinomycetes contributing in production of bioactive compound with antibiofilm capacity.
b. The present study will be helpful in determination of mechanism of action of bioactive compound against bacterial biofilms.
c. The present study will be helpful in production of bioactive compound which will contribute in recent drug screening program to act against drug resistant bacteria.

6. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved

First year (2019-2020):-

- Completion of course work and Literature survey
- Sample collection
- Isolation of Actinomycetes

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Second year (2020-2021): -

- Screening of isolated actinomycetes for bioactive metabolite production and antibiofilm potential.
- Production of bioactive compound
- Purification and Characterization of produced bioactive compound
- Study of antimicrobial efficacy of agents against biofilm producing Bacteria.
- Practice on writing skill the research article by referring th research paper of various reputed journals.

Third year (2021-2022): -

- Quantification of its antibiofilm activity
- Preparation and submission of research article to the reputed national and international journals.
- Compilation of data for thesis writing
- Submission of thesis to the university

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